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Short communication

Isolation of a new polymorphic TC maize microsatellite located on the long arm of chromosome 10

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Abstract

Simple sequence repeats (SSR), also called microsatellites, were previously proved to be an important class of DNA markers. The isolation, mapping and designing of primers to the flanking regions of a new maize SSR is reported. The new marker, with a core motif of (TC)12, designated as MZETC34, was mapped to the long arm of chromosome 10.

Microsatellites, also called simple sequence repeats (SSR), have been described as 'second generation', polymorphic DNA markers [5]. They have been recommended as the standard markers used to prepare highly saturated genetic maps of any eukaryote genome [2]. A map based on SSR consists of a list of oligonucleotide sequences. Once published, an SSR becomes available to a wide range of potential users, and does not require shipping or storing of cloned probes. Akkaya et al. [1] assessed SSR length polymorphism in the soybean (*Glycine max* (L.) Merr.) and, for the first time, proved the usefulness of this marker in a higher plant. The presence, informativeness and wide application of SSRs have, since, been proven in other plant species including rice [12, 13], *Brassica napus* [7], barley [9], sunflower [4], *Arabidopsis thaliana* [3] and wheat [6]. The first six SSRs in maize were described and mapped by Senior and Heun [10].

Here, we report the isolation, mapping and designing of primers to the flanking regions of a new maize microsatellite, MZETC34, with a core motif (TC)12. The microsatellite was isolated from a genomic library prepared using DNA from the inbred line J105. *Sau3AI* fragments, 200–400 bp long, were cloned in the pBluescript II KS+ plasmid vector (Stratagene, La Jolla, CA). The library was screened as described by Akkaya et al. [1] using (CT)8 probe, and a TC positive clone designated MZETC34 was selected and sequenced. Unique primers designed to the flanking regions of the (TC)12 core element (Figure 1) were used to amplify corn DNA by the polymerase chain reaction (PCR), producing an amplification product which was 104 bp long in the J105 inbred line. Nine length alleles were detected when these primers were screened on 19 maize genotypes and analyzed on a sequencing gel. The inbred lines IL731A and W6786 [11] were among the polymorphic genotypes. The amplification product of IL731A was 100 bp long while that of W6786 was 104 bp long (Figure 1).

Seventy seven $F_{2:3}$ families of the cross IL731a × W6786 were scored for MZETC34 segregation (Figure 1). Twenty one were homozygotes carrying the 100 bp allele, 34 were heterozygotes, and 22 were homozygotes carrying the 104 bp allele. The results fitted the 1:2:1 ratio of codominant segregation with a χ^2 value of 1.08 ($P = 0.583$). The sweet corn linkage map was recently established using this population [11]. In order to localize MZETC34 on the sweet corn map, the linkage relationships between MZETC34 and 91 RFLP markers were analyzed with MAPMAKER [8] using a LOD score of 3.0 and a recombination fraction of 0.4. Employing this procedure MZETC34 was located on the long arm of chromosome 10, situated between the RFLP markers umc 152 and npi 582 (Figure 2).

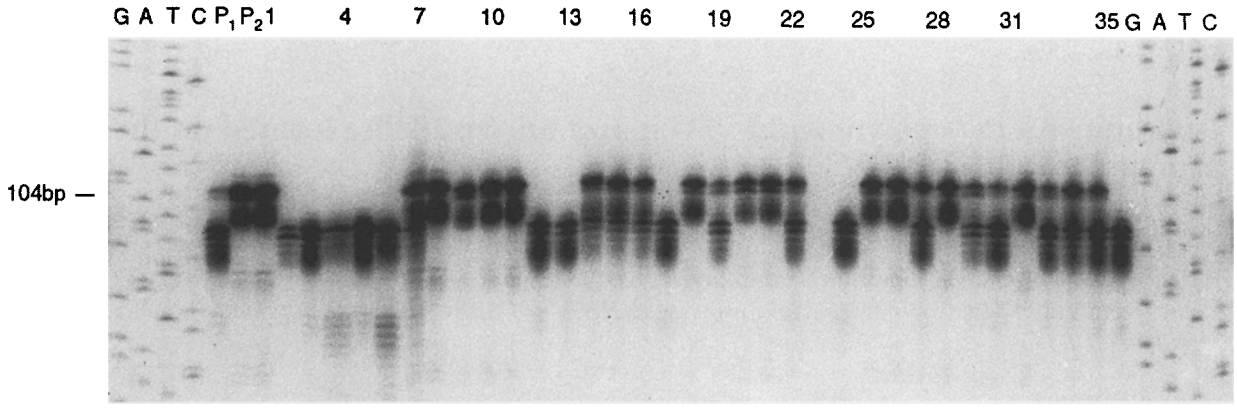

Figure 1. Radiolabelled PCR products amplified in the F_2 progeny of the cross IL731A × W6876. P_1: IL731A; P_2: W6876; lanes 1–35: F_2 plants. The sequencing ladder is M13mp18 ssDNA (GATC). Amplification primers flanking the (TC)12 motif: CCAAAACACAAGTCTGAACTC (forward); CTGTGAAAGGGATGCAAG (reverse).

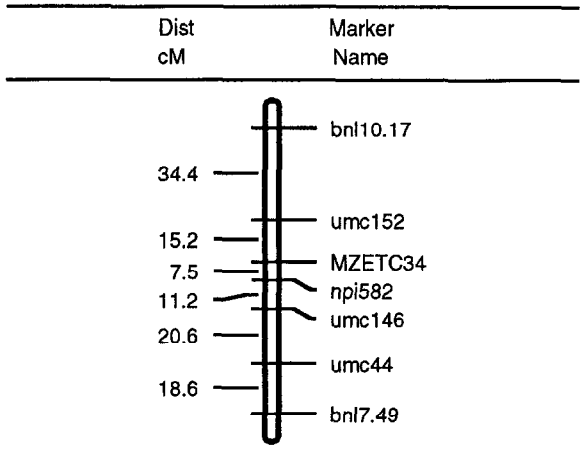

Figure 2. A linkage map of RFLP and the MZETC34 SSR on chromosome 10.

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